Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes

Amino Acid Ingestion Strongly Enhances Insulin Secretion in Patients With Long-Term Type 2 Diabetes

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OBJECTIVE — Insulin secretion in response to carbohydrate intake is blunted in type 2 diabetic patients. However, it is not clear whether the insulin response to other stimuli, such as amino acids, is also diminished. Recently, we defined an optimal insulinoptropic mixture containing free leucine, phenylalanine, and a protein hydrolysate that substantially enhances the insulin response in healthy young subjects when coingested with carbohydrate. In this study, we aimed to investigate the insulinotropic capacity of this mixture in long-term type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — Ten type 2 diabetic patients (aged 59.1 ± 2.0 years, BMI 26.5 ± 0.7 kg/m^2) and 10 healthy control subjects (58.8 ± 2.1 years, 26.5 ± 0.7 kg/m^2) visited our lab twice, during which insulin responses were determined following ingestion of carbohydrate only (CHO) or carbohydrate with the free amino acid/protein mixture (CHO+PRO). All subjects received 0.7 g·kg^{-1}·h^{-1} carbohydrate with or without 0.35 g·kg^{-1}·h^{-1} of the amino acid/protein mixture.

RESULTS — Insulin responses were dramatically increased in the CHO+PRO trial in both the type 2 diabetic and control groups (189 and 114%, respectively) compared with the CHO trial ($P < 0.01$). Plasma glucose, glucagon, growth hormone, cortisol, IGF-I, and IGF binding protein 3 responses were not different between trials within the 2-h time frame.

CONCLUSIONS — The insulin secretory capacity in long-term type 2 diabetic patients is substantially underestimated, as the insulin response following carbohydrate intake can be nearly tripled by coingestion of a free amino acid/protein mixture. Future research should be performed to investigate whether such nutritional interventions can improve postprandial glucose disposal.

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The synergistically stimulating effect of the combined intake of carbohydrate and protein on plasma insulin concentration was first reported in the 1960s (1,2) and later confirmed in both healthy (3) and type 2 diabetic subjects (4–6). In addition, an increase in plasma insulin concentration has been reported following the intravenous infusion of free amino acids in both healthy (7–9) and type 2 diabetic subjects (10). In accordance, various in vitro studies using incubated β-cells of the pancreas have described strong insulinotropic effects of arginine, leucine, and phenylalanine (11–18). Recently, we performed a series of studies in which we determined the in vivo insulinotropic potential of various free amino acids and protein (hydrolysates) when ingested in combination with carbohydrates in healthy young subjects (19–21). In trying to define an optimal insulinotropic amino acid and/or protein (hydrolysate) mixture, when coingested with a carbohydrate solution, we found a mixture containing wheat protein hydrolysate, free leucine, and phenylalanine to be most potentiating (20,21).

Maximizing insulin secretion by such a nutritional intervention has recently regained much attention because it has been proven to be a practical means to accelerate postexercise muscle glycogen resynthesis (19,22) and increase net muscle protein anabolism (23,24). Clearly, maximizing endogenous insulin secretion by the combined ingestion of carbohydrate and an insulinotropic amino acid/protein (hydrolysate) mixture could be of particular significance in type 2 diabetic patients. From one perspective, increasing postprandial endogenous insulin secretion could help to increase plasma glucose disposal and improve glucose homeostasis, thereby potentially postponing the patient's dependency on exogenous insulin therapy. In addition, administration of carbohydrate, free amino acids, and protein hydrolysates, leading to both hyperinsulinemia and hyperaminoacidemia, has been shown to stimulate protein synthesis and inhibit proteolysis (25,26). The latter is of particular interest, as muscle protein breakdown rates are markedly elevated in uncontrolled type 2 diabetes (27).

In type 2 diabetes, as well as in a state of impaired glucose tolerance with otherwise normal fasting glucose concentrations, insulin secretion shows several abnormalities (28). These secretory defects include reduced early insulin secretory response to oral glucose, reduced ability of the β-cell to compensate for the degree of insulin resistance, decreased glucose-sensing ability of the β-cell, and shifts to the right in the dose-response curves relating glucose and insulin secretion, which is indicative of a progressive insensitivity of the β-cell to glucose (28).

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Abbreviations: GH, growth hormone; IGF-BP3, IGF binding protein 3; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Subjects' characteristics*

	Control group	Type 2 diabetes
n	10	10
Age (years)	58.8 ± 2.1	59.1 ± 2.0
Body weight (kg)	83.4 ± 2.7	82.0 ± 1.8
Height (m)	1.77 ± 0.01	1.76 ± 0.02
BMI (kg/m^2)	26.5 ± 0.74	26.5 ± 0.71
Fasting blood glucose (mmol/l)	5.3 ± 0.1	11.4 ± 1.3*
Blood glucose OGTT (120 min)	4.1 ± 0.3	19.5 ± 1.1*
Type 2 diabetes duration (years)	—	8.9 ± 1.5

Data are means ± SE. *Significantly different from control group ($P < 0.01$).

All of these defects involve glucose-sensing and -signaling pathways in the β-cell. Although insulin secretion in response to the prevailing glucose concentration may be blunted in type 2 diabetic subjects, it could be hypothesized that insulin secretion in response to other stimuli remains unaffected. As mentioned above, arginine, leucine, and phenylalanine have been shown to be potent stimuli for insulin secretion in the β-cell (10–18). In accordance, we have shown that the in vivo insulinotropic potential of the combined ingestion of carbohydrate with protein (hydrolysate) can be enhanced by the addition of free leucine and phenylalanine (20,21). However, the insulinotropic capacity of oral supplementation with such a free amino acid–containing mixture has not been investigated in type 2 diabetic patients. As hyperglycemia is no longer accompanied by compensatory hyperinsulinemia in long-term type 2 diabetic patients, it is often assumed that the insulin secretory capacity of the β-cells is substantially reduced in these patients. Therefore, we aimed to investigate whether endogenous insulin secretion after carbohydrate intake can be increased by coingestion of a free amino acid/protein hydrolysate mixture in long-term type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

Subjects

Ten long-term diagnosed male type 2 diabetic patients were selected to participate in this study. Exclusion criteria were impaired renal or liver function, obesity (BMI >30 kg/m^2), cardiac disease, hypertension, diabetes complications, and exogenous insulin therapy. Most subjects (n = 8) were using oral antidiabetic agents (mainly sulfonylureas). Ten healthy, normoglycemic, male adults, matched for age and BMI, participated as control subjects. In the diabetic subjects, blood glucose–lowering medication was witheld for 3 days before participation in the trials and throughout the entire experimental period. Subjects were screened for glucose intolerance/type 2 diabetes by an oral glucose tolerance test (OGTT) according to the World Health Organization criteria of 1999 (29). Subjects' characteristics are provided in Table 1. All subjects were informed about the nature and risks of the experimental procedures before their informed consent was obtained. All clinical trials were approved by the local Medical Ethical Committee.

Pretesting

Before selection in the study, all subjects performed an OGTT. After an overnight fast, subjects reported to the lab and a blood sample was collected. Thereafter, a bolus of 75 g glucose (dissolved in 250 ml water) was ingested, and 2 h later a second blood sample was obtained (Table 1).

Study design

Each subject participated in two trials, separated by 1 week, in which the insulin response to the ingestion of two drink compositions (carbohydrate only [CHO] or carbohydrate plus the free amino acid/protein mixture [CHO+PRO]) was determined. Subjects were seated and remained inactive during both 3-h trials. Drinks were provided in randomized order and double-blind fashion. Beverages were vanilla flavored to make the taste comparable in all trials. Subjects were instructed to refrain from heavy physical labor and to keep their diet as constant as possible for 2 days before the trials.

Protocol

After an overnight fast, subjects reported to the laboratory at 0830. A teflon catheter (Baxter BV, Utrecht, the Netherlands) was inserted into an antecubital vein, and a resting blood sample was drawn (0 min). Immediately thereafter, subjects drank an initial bolus (3 ml/kg) of a given test drink (CHO or CHO+PRO trial). Repeated boluses (3 ml/kg) were ingested every 30 min until 90 min. Blood samples were drawn at 15-min intervals for measurement of plasma glucose and insulin concentrations. In addition, growth hormone (GH) and cortisol concentrations were determined at 0, 60, and 120 min. IGF-I and IGF binding protein 3 (IGFBP3) concentrations were determined at 0 and 120 min. In four subjects within each group, additional blood samples were collected at 2, 4, 6, 8, and 10 min for insulin and glucose analysis.

Beverages

At 0, 30, 60, and 90 min, subjects received a beverage volume of 3 ml/kg to ensure a given dose of 0.7 g · kg^{-1} · h^{-1} carbohydrate (50% glucose and 50% maltodextrin) with or without an additional 0.35 g · kg^{-1} · h^{-1} of an amino acid/protein hydrolysate mixture (CHO+PRO or CHO trial, respectively). This hydrolysate/amino acid mixure consisted of a wheat protein hydrolysate (50%, 0.17 g · kg^{-1} · h^{-1}), free leucine (25%, 0.09 g · kg^{-1} · h^{-1}), and free phenylalanine (25%, 0.09 g · kg^{-1} · h^{-1}). Glucose and maltodextrin were obtained from AVEBE (Veendam, the Netherlands), crystalline amino acids from BUFA (Uitgeest, the Netherlands), and the wheat protein hydrolysate was prepared by Quest International (Naarden, the Netherlands). Both drinks were uniformly flavored by adding 0.2 g sodiumsaccharinate, 1.8 g citric acid, and 5 g cream vanilla flavor (Quest International) per liter of beverage.

Analysis

Blood (10 ml) was collected in EDTA-containing tubes and centrifuged at 1,000g and 4°C for 10 min. Aliquots of plasma were immediately frozen in liquid nitrogen and stored at −80°C. Glucose (Uni Kit III, 07367204; Roche, Basel) was analyzed with the COBAS FARA semiautomatic analyzer (Roche). Plasma insulin (Insulin RIA 100 kit; Pharmacia, Uppsala, Sweden) and glucagon (DPS, Los Angeles, CA) concentrations were analyzed

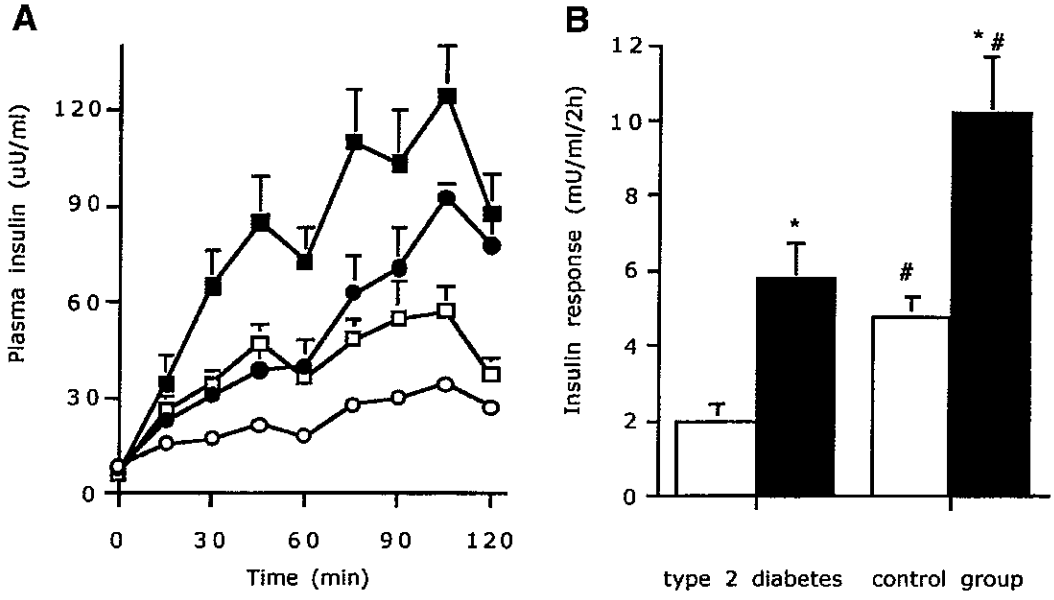

Figure 1—*Plasma insulin concentration (A) and response (B) over a 2-h period following the ingestion of carbohydrate (○/□) or carbohydrate with a protein hydrolysate/amino acid mixture (●/■) in subjects with type 2 diabetes (○/●, n = 10) and healthy control subjects (□/■, n = 10). Data are expressed as means ± SE. *Significant difference between CHO and CHO+PRO trial within each group; #significant difference between groups in the CHO/CHO+PRO trial.*

by radioimmunoassay. Sequential immunometric assays were used to determine plasma GH (Nichols Institute Diagnostics, San Juan Capistrano, CA) and cortisol (Chiron Diagnostics, East Walpole, MA) concentrations. Plasma IGF-I was performed using an immunoradiometric assay (DSL-5600 ACTIVE; DSL Deutschland, Sinsheim, Germany) with an intraassay variance of 3.0% and an interassay variance of 1.5%. Plasma IGF-BP3 concentrations were determined by another immunoradiometric assay (DSL-6600 ACTIVE; DSL Deutschland) with an intraassay variance of 3.2% and an interassay variance of 0.5%.

Statistics

Data are expressed as means ± SE. The plasma responses were calculated as areas under the curve above baseline values. Statistical analysis of the data were performed using multiway ANOVA. Changes in time within each group were checked for statistical significance using repeated-measures ANOVA. Differences between trials were determined for statistical significance using the Scheffé's post hoc test ($P < 0.05$).

RESULTS— In both the CHO and CHO+PRO trials, fasting plasma insulin concentrations (0 min) were similar between groups. In all trials, plasma insulin concentrations increased significantly during the first 105 min, after which levels tended to decrease (Fig. 1*A*). After expressing the insulin response as area under the curve (above baseline values), we observed significant differences between both trials and groups (Fig. 1*B*). Coingestion of the protein hydrolysate/amino acid mixture (CHO+PRO) resulted in a substantial increase in insulin response that was an additional 189 and 114% higher than the ingestion of carbohydrates only (CHO) in the type 2 diabetic and healthy control subjects, respectively ($P < 0.01$). The additional increase in insulin response in the CHO+PRO trial was not significantly different between groups (3.77 ± 0.53 and 5.43 ± 1.03 mU · ml^{-1} · 2 h^{-1}, respectively). The insulin responses in both the CHO and CHO+PRO trials were significantly lower in the type 2 diabetic subjects than in the normoglycemic control subjects ($P < 0.01$).

Fasting plasma glucose concentrations were substantially higher in the type 2 diabetic subjects than in the normoglycemic control subjects (11.6 ± 0.7 and 5.0 ± 0.1 mmol/l, respectively, $P < 0.01$). Plasma glucose concentrations increased from 0 min onward in the type 2 diabetic subjects in both the CHO and CHO+PRO trials and tended to plateau over the last 30 min of the 2-h period (Fig. 2*A*). No significant differences were observed within this 2-h time frame between the different trials in the diabetic group. In the normoglycemic control subjects, plasma glucose concentrations increased over the first 30–45 min, after which concentrations returned to baseline values (Fig. 2*A*). Though plasma glucose concentrations tended to be lower between 45 and 90 min in the CHO+PRO trial, no significant differences were observed between the CHO and CHO+PRO trials within this 2-h time frame ($P = 0.06$).

Plasma glucose concentrations remained substantially higher in the type 2 diabetic patients than in the control group in both the CHO and CHO+PRO trials ($P < 0.01$). After expressing the plasma glucose response as area under the curve (above baseline values), we observed no significant differences between trials in each group (Fig. 2*B*). However, both the glucose responses in the CHO and CHO+PRO trial were significantly higher in the type 2 diabetic subjects than in the normoglycemic control subjects ($P < 0.01$). In four subjects in each group, additional blood samples were taken at 2, 4, 6, 8, and 10 min to check for the potential prevalence of a primary secretory insulin response. However, on a whole-

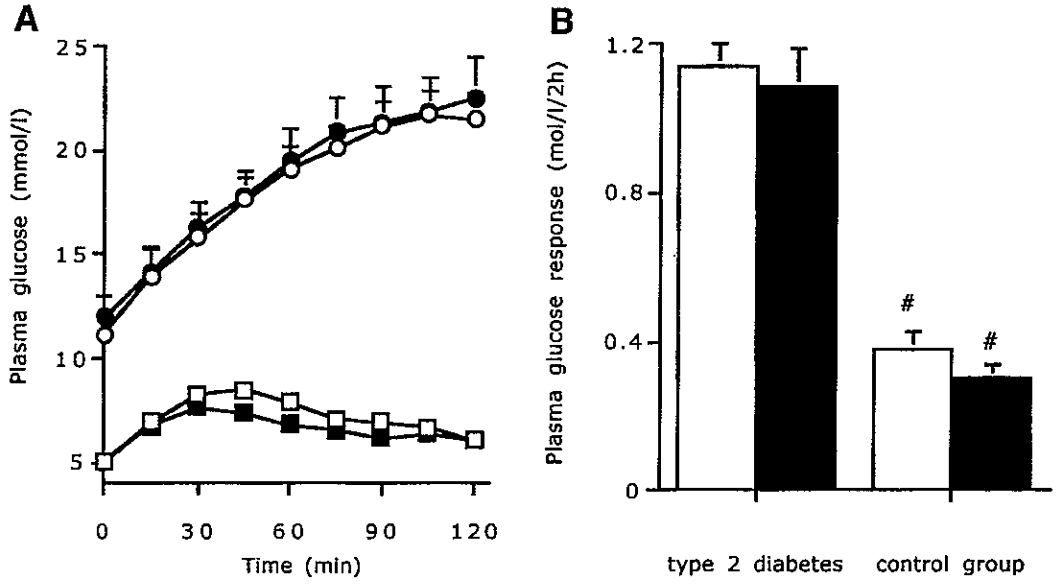

Figure 2—*Plasma glucose concentration (A) and response (B) over a 2-h period following the ingestion of carbohydrate (○/□) or carbohydrate with a protein hydrolysate/amino acid mixture (●/■) in subjects with type 2 diabetes (○/●, n = 10) and healthy control subjects (□/■, n = 10). Data are expressed as means ± SE. #Significant difference between groups in the CHO/CHO+PRO trial.*

body level, we were unable to demonstrate such a response; both glucose and insulin concentrations increased during the first 10 min after ingestion of the first beverage but remained below the values recorded at 15 min (data not shown).

In both groups, plasma glucagon concentrations increased over time in all trials ($P < 0.05$) (Table 2). No significant differences were observed between groups. Within groups, a stronger increase in plasma glucagon in the CHO+PRO than the CHO trial was observed in the control group only ($P < 0.05$). Fasting plasma GH and cortisol concentrations tended to be higher in the type 2 diabetic subjects than in the control subjects ($P = 0.10$ and $P = 0.14$, respectively). In both the type 2 diabetic and control subjects, plasma GH concentrations decreased over time in both the CHO and CHO+PRO trials (Ta-

Table 2—*Plasma concentrations*

Time	Group	Trial	0 min	60 min	120 min
Glucagon (pmol/l)	Type 2 diabetes	CHO	9.3 ± 0.7	12.6 ± 1.2	14.4 ± 1.6*
	Control group	CHO	13.1 ± 1.5	13.6 ± 1.1	15.5 ± 1.2*
	Type 2 diabetes	CHO+PRO	11.5 ± 1.5	15.7 ± 2.2	15.9 ± 1.8*
	Control group	CHO+PRO	14.0 ± 1.5	16.9 ± 1.5	19.5 ± 1.3*†
Growth hormone (μg/l)	Type 2 diabetes	CHO	0.75 ± 0.27	0.49 ± 0.31	0.08 ± 0.03
	Control group	CHO	0.49 ± 0.23	0.30 ± 0.18	0.07 ± 0.02
	Type 2 diabetes	CHO+PRO	1.02 ± 0.36	0.23 ± 0.11	0.18 ± 0.08*
	Control group	CHO+PRO	0.39 ± 0.20	0.16 ± 0.08	0.11 ± 0.05
Cortisol (nmol/l)	Type 2 diabetes	CHO	465 ± 27	270 ± 16	238 ± 18*
	Control group	CHO	434 ± 33	284 ± 21	270 ± 24*
	Type 2 diabetes	CHO+PRO	453 ± 25	332 ± 40	329 ± 33*
	Control group	CHO+PRO	406 ± 19	302 ± 29	278 ± 25*
IGF-I (nmol/l)	Type 2 diabetes	CHO	21.7 ± 3.4	—	19.4 ± 3.5
	Control group	CHO	23.5 ± 3.1	—	22.0 ± 2.8
	Type 2 diabetes	CHO+PRO	19.0 ± 2.9	—	19.5 ± 3.2
	Control group	CHO+PRO	20.9 ± 2.5	—	21.0 ± 2.2
IGF-BP3 (nmol/l)	Type 2 diabetes	CHO	126 ± 8	—	—
	Control group	CHO	129 ± 7	—	—
	Type 2 diabetes	CHO+PRO	125 ± 9	—	—
	Control group	CHO+PRO	127 ± 5	—	—

Data are means ± SE. *Significant change over time ($P < 0.05$). †Significant difference between trials within the separate groups. There were no significant differences in plasma glucagon, cortisol, and GH between the groups.

ble 2). However, due to large variances in plasma GH concentrations, this time effect only reached statistical significance in the CHO+PRO trial in the diabetic group ($P < 0.05$). Plasma cortisol concentrations significantly decreased over time in both groups within both trials ($P < 0.05$). Further analysis of plasma GH, cortisol, IGF-I, and IGF-BP3 concentrations did not reveal any significant differences between groups or between trials. In four diabetic patients and four control subjects, IGF-BP3 concentrations were determined at several points in time, which did not show any change (data not shown).

CONCLUSIONS — The diabetic patients selected to participate in this study had been diagnosed with type 2 diabetes for >8 years and were about to switch from taking oral antidiabetic agents only (mainly sulfonylureas) to starting exogenous insulin therapy. In the selected patients, hyperinsulinemia, as a compensatory effect to reduce prevailing hyperglycemia, was not present anymore. Fasting plasma insulin concentrations were not different from values observed in the control group (Fig. 1), but glucose concentrations were substantially higher (Table 1 and Fig. 2). The OGTT revealed substantial glucose intolerance in the type 2 diabetic group (Table 1).

As hyperglycemia is no longer accompanied by compensatory hyperinsulinemia in long-term type 2 diabetic patients, it is often assumed that the insulin secretory capacity of the β-cells is substantially reduced. However, our data argue against this assumption. The insulin response following carbohydrate intake only (CHO) was less than half in the type 2 diabetic patients compared with the normoglycemic control subjects ($P < 0.01$) (Fig. 1) and clearly demonstrates the reduced sensitivity of the β-cell to glucose. However, the insulin response in these patients could be substantially increased (by an additional 189%) by coingestion of the amino acid/protein mixture ($P < 0.01$). In the control group, insulin responses increased by 114% in the CHO+PRO trial ($P < 0.01$). The additional increase in insulin response following coingestion of the free amino acid/protein mixture was not significantly different between groups, which seems to suggest that even in these long-term type 2 diabetic patients, insulin secretion in response to other stimuli is not impaired.

The increase in insulin concentration following the combined ingestion of carbohydrate with leucine, phenylalanine, and a protein hydrolysate has been shown to be highly correlated with the increase in plasma leucine and phenylalanine concentration (21). Substantial progress has recently been made in the understanding of the mechanisms and signaling pathways by which leucine (12,15–17), arginine (13–16,30), and phenylalanine and its derivatives (18) stimulate β-cell function and insulin secretion in in vitro studies using isolated β-cells and insulin-secreting pancreatic β-cell lines. Clearly, the large responsiveness to the coingestion of the insulinotropic amino acid/protein mixture in the present study strongly suggests that amino acids can provide a glucose-independent stimulus to the pancreatic β-cells that is preserved in patients with long-term type 2 diabetes.

Although insulin responses were strongly increased in the CHO+PRO trials, the mean glucose responses were not significantly reduced within this time frame, neither in the type 2 diabetic patients nor in the healthy control subjects (Fig. 2). This can be explained by the fact that in the present study, repeated boluses of a carbohydrate-containing solution were provided. The latter resulted in a substantial amount of carbohydrate being ingested over a relatively short period. Therefore, it is more than likely that a potential increase in plasma glucose disposal is being obscured by accelerated intestinal glucose absorption. Other studies with longer time frames have reported a plasma glucose–lowering effect after coingestion of protein and/or amino acids with carbohydrates, which is in line with our findings. For example, Gannon et al. (5) demonstrated an increase in insulin secretion following the combined ingestion of a single bolus of glucose with intact proteins (both egg protein and cottage cheese) in type 2 diabetic subjects and reported an 11 and 20% decrease in glucose area response over a 5-h postprandial period. The plasma glucose–lowering effect in that study became apparent mainly during the last 3 h. In accordance, we have reported a significant decrease in glucose response (−48%) following the coingestion of the applied amino acid/protein mixture in trained cyclists over a 5-h postexercise period (19).

Theoretically, the insulinotropic mixture used in the present study might stimulate glucagon secretion, as purified pancreatic α-cells have been shown to secrete glucagon in response to an amino acid mixture (31). To determine whether increased glucagon secretion in the CHO+PRO trials could have accelerated hepatic glycogen breakdown and thus contributed to the lack of a plasma glucose–lowering effect within the 2-h time frame in both groups, we also measured plasma glucagon concentrations (Table 2). However, a significant difference in the increase in plasma glucagon concentration between trials was observed within the control group only. The absence of such an effect in the type 2 diabetic group may partly be explained by the prevailing hyperglycemia, because high glucose concentrations have been shown to inhibit amino acid–induced glucagon release through a direct insulin-independent action on the α-cells (31). Though no significant change in the 2-h glucose response was observed in either the diabetic or control group, this does not necessarily imply that plasma glucose disposal was not improved, as explained above. Clearly, further research is needed to establish the magnitude to which free amino acid/protein supplementation can improve glucose disposal and reduce plasma glucose concentrations over time. Subsequently, it should also be investigated whether such dietary supplements can increase the efficiency of traditional glucose-lowering medication and/or provide an effective and practical means to reduce, delay, or even prevent the diabetic patients' dependency on conventional blood glucose–lowering medication and/or exogenous insulin administration.

Because of the proposed physiological role of GH and IGF-I in insulin sensitivity (32), we also investigated direct or indirect effects of oral free amino acid/protein supplementation on plasma GH, cortisol, IGF-I, and IGF-BP3 concentrations in both the type 2 diabetic patients and normoglycemic control subjects (Table 2). However, no significant differences in plasma GH, cortisol, IGF-I, or IGF-BP3 were observed between trials or groups.

In conclusion, the insulin secretory reponse following carbohydrate ingestion is substantially impaired in long-term type 2 diabetic patients. However, the insulin response can be substantially increased by the coingestion of a free amino acid/protein mixture. Because the applied

amino acid/protein mixture, containing free leucine, phenylalanine, and an ample source of essential amino acids, has a high insulinotropic potential in type 2 diabetic patients, future research is warranted to investigate the use of such amino acid–containing mixtures as tools to increase postprandial glucose disposal and improve glucose homeostasis.

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